In this presentation we illustrate in detail the folding mechanism of a prototypical beta-hairpin, namely the C-terminal fragment of protein GB1, by means of all-atom molecular dynamics (MD) simulations in explicit solvent, using metadynamics to accelerate the sampling of standard MD and reconstruct the free energy of the process.
Our results show clearly that the unfolded ensemble of this protein does not comply with the classical view of a collection of disordered coil conformations. Indeed we found out that a fully stretched configuration is unstable towards the formation of a turn in the central region of the peptide. This loop can assume two different conformations: a native-like one, which eventually leads to the 2:4 native structure, and a non-native turn which characterizes the ensemble of the unfolded states among which an ordered 3:5 misfolded structure is particularly stable.
Our results, corroborated by several experiments, support the growing idea that the unfolded ensemble of proteins and even of small polypeptides can be characterized by some form of non-native structure.

404-Pos Board B283
Beta-barrel Proteins that Reside in the E. coli Outer Membrane In Vivo Demonstrate Varied Folding Behavior In Vitro
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Little is known about the dynamic process of membrane protein folding, and few models exist to explore it. We have doubled the number of *Escherichia coli* outer membrane proteins (OMPs) for which folding into lipid bilayers has been systematically investigated. We cloned, expressed, and folded nine OMPs: outer membrane protein X (OmpX), OmpW, OmpA, the *crcA* gene product (PagP), OmpT, outer membrane phospholipase A (OmpLa), the *fadl* gene product (FadL), the *yaet* gene product (Omp85), and OmpF. These proteins share a transmembrane β-barrel motif, but vary in barrel size and primary sequence. We quantified their ability to fold into a matrix of bilayer environments by SDS-PAGE. Several trends emerged from these experiments: higher pH values, thinner bilayers, and increased bilayer curvature promote folding of all OMPs. Increasing the incubation temperature promoted folding of several OMPs but inhibited folding of others. We discovered that OMPs do not have the same ability to fold into any single bilayer environment. We show that while environmental factors are influential, OMP folding must also be modulated by intrinsic protein properties. To rationalize the differences in folding results between OMPs, we explored their kinetic profile and determined their resistance to thermal denaturation. Although these proteins share a common structural motif and have evolved to reside in the same bilayer environment *in vivo*, we found that these OMPs vary in their folding behavior.

405-Pos Board B284
Bistable Entropy Landscape of Sequences and Folds of Proteins
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The evolutionary capacity of a protein fold is defined as the number of sequences that match a particular structure. The logarithm of the evolutionary capacity is proportional to the sequence entropy. We computed the sequence entropies for a representative set of protein structures from the Protein Data Bank (1590 folds). For each of the structures we sample about ten million sequences acceptable to the target fold, and estimate the evolutionary capacity for a range of energies with telescoping ratios. The calculations are conducted with three empirical energy functions that were designed for different tasks in computational biology. The probability of observing a protein with a capacity normalized with respect to length is doubly peaked. The proteins at the peak of lower capacity are most likely to belong to the SCOP classes of all-alpha and the alpha/beta classes. The other peak of capacity includes structures from all-beta and the alpha+beta classes similarly to the overall distributions. Experimentally determined mutants of each protein in the set were collected using BLAST with E-value cutoff of 10^{-10}. The evolutionary capacity and experimentally determined number of mutants are positively correlated for proteins at the peaks. Among all the proteins within the two peaks, a HIV reverse transcriptase at the higher peak has the maximum number of mutants. HIV virus is known for its high evolution rate, which corresponds to high evolutionary capacity in our research. We also examine the network in which sequences flip between alternative folds. The network density and dynamics will be reported.

406-Pos Board B285
Amyloid β Proteins, Modified by a Lipid Oxidation Product, Are Nucleation Sites for Fibril Formation on Lipid Membranes
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Oxidatively damaged lipid membranes are known to promote the aggregation of amyloid β (Aβ) proteins and fibril formation. When lipid membranes contain ω-6 polyunsaturated fatty acyl chains, 4-hydroxy-2-nonenal (HNE) is produced during oxidative stress. We previously demonstrated that HNE modifies the three His residues in Aβ proteins by Michael addition, which increases the hydrophobicity and affinity of Aβ proteins for the membrane surface, and promotes the aggregation of unmodified Aβ proteins into fibrils. There are two different mechanisms by which the promotion of fibril formation may occur. HNE-modified Aβ proteins may act as catalytic templates that stabilize monomers in a fibrillar conformation, but do not ultimately become a part of the fibril (template mechanism). Alternatively, they may act as seeds that reduce a kinetic barrier to adopt a fibrillar conformation by becoming a part of the fibril (seed mechanism). In this report, the concentrations of HNE-modified Aβ proteins in fibrils, in solution, and in lipid vesicle membranes were monitored in order to distinguish between these two mechanisms.
Results indicate that HNE-modified Aβ proteins associate primarily with lipid membranes, suggesting that HNE promotes fibril formation by the template mechanism, and that relatively little HNE-modified Aβ is incorporated into fibrils. This result is pathophysiologically significant because such templates are not consumed by the process of fibril formation and may be long-lived. The formation of isolated templates may also account for the patchy distribution of amyloid fibril plaques in brain tissue afflicted with Alzheimer's disease.

407-Pos Board B286
Rescuing Functional Protein from Amyloid-Like Structure
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Formation of amyloid deposits is the molecular background of several diseases. Protein oligomers and aggregates formed in the process are connected to the observed pathogenesis. Amyloids represent the end stage of a multi-step aggregation cascade. Although some recovery of the enzyme activity from amyloid deposits has been reported in the case of the lysozyme (Booth II et al., Nature 385 (1997) 787-93), the recovery of the active structure from amyloid or amyloid-like deposits has not been studied. Here we show that phosphoglycerate kinase can be refolded into the biologically active structure from amyloid-like fibrils. First, amyloid-like fibrils were grown from phosphoglycerate kinase. The conversion of the protein structure was confirmed by electron microscopy, enzyme activity assays, as well as by Congo red and Thioflavin T binding measurements. Next, the protein was refolded into its native structure. Biological equivalence of the reference and recovered enzyme was confirmed by enzyme activity and differential scanning calorimetry measurements. We found that stabilizing the native fold is not enough for the efficient recovery of the native enzyme. The aggregates have to be destabilized before the formation of the native structure is initiated.

408-Pos Board B287
Dissecting the N-terminal Helical Domain of Apolipoprotein B
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High levels of low density lipoprotein (LDL, the bad cholesterol) are associated with cardiovascular disease, the leading cause of death in western countries. Very low density lipoprotein (VLDL, the precursor to LDL) secretion requires proper folding of the N-terminal domains of apolipoprotein B (ApoB). The N-terminal domain of ApoB is homologous to lipovitellin, whose structure is known. It is composed of a β-barrel, followed by a helical domain and two β-sheet domains. There is little direct structural information on any part of ApoB. Structural studies of the N-terminal domain of ApoB are complicated by aggregation in the absence of lipids. Our initial goal was to isolate an independent folding domain suitable for study by NMR. Two constructs encoding ApoB6.4-8 and ApoB6.4-9 were found to be cooperatively folded and show helical CD spectra. Single point mutations (e.g. L343V) within this region of ApoB have been shown to cause Familial Hypobetalipoproteinemia (FHBL). FHBL is characterized by low levels of plasma ApoB-containing lipoproteins. These mutations result in retention and degradation of ApoB in the ER. Despite the conservative mutation, our hypothesis is that the mutations cause a structural defect in this helical domain which disrupts efficient secretion of VLDL.

409-Pos Board B288
Peptide Structure Stabilization: A Study Of Aromatic-aromatic Interaction And pH Effect On A β-hairpin Stability
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Aromatic side chains in proteins are often involved in aromatic pairs, most of which form interacting networks of three or more aromatic side chains. (Petsko